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Biotechnological Uses in Assessment of Some Meat Products Adulteration with Equine Meat

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ABSTRACT

Meat adulteration constitutes an important problem in Egypt. Adulteration of meat may occur by substitution of low priced or even banned meat species for that high priced one. In this study, polymerase chain reaction (PCR) techniques were applied for detection of different meat products adulteration with equine meat by using equine specific primers. One hundred samples from different meat products (luncheon, hot dog, Sausage and minced meat) 25 of each were collected randomly from popular areas in Alexandria city. Samples were considered positive when a single band of DNA at 439 bp were evident in the ethidium bromide stained gels compared with the known molecular DNA size marker. The results obtained revealed that the incidence of adulteration in luncheon, hot dog, sausage and minced meat were 6 (24%), 2 (8%), 16 (64%) and 14 (56%), respectively. It is concluded that PCR could be useful for fast, easy, and reliable control of adulterated consumer raw and processed meat products with equine meat

1. INTRODUCTION

In the last few decades, adulteration of meat products has become a considerable problem in many countries over and above Arabian countries. Meat species specification is an utmost important field of food forensics. It is more challenging and revolutionary task to ensure the quality of meat and help in conservation of law existing in different countries (Singh and Sachan 2009). The ability to detect less desirable or objectionable species in meat products is important not only for economic, health, religious and ethical reasons, but also to ensure fair trade and compliance with legislation (Nakyinsige et al., 2012).

Meat identification in various foods including processed meat products deserves an increasing interest owing to many considerations. Rapid examination of adulteration are very critical issues for healthical requirements, specific food allergies, religious affairs, fraud and malicious marketing practices in addition to economic and legal concerns (Mane et al., 2009).

The adulteration of inferior quality meat into superior quality meat is a common practice all over the world (Hou et al., 2015). Minced meat productions remove the morphological characteristics of muscle, making it difficult to identify one type of muscle from another. Because after grinding and mixing, the origin of meat species is easy to conceal in the mixture due to the change of meat texture, colour and appearance or even flavour (Manjula et al., 2009). For this reason, meat substitution with unspecified species, usually of lower quality, is the most common form of economic adulteration in the minced meat industry, constituting a fraudulent act that could have economic and health repercussions.

Problems related to adulteration of meat species in ground and comminute products have been a widespread problem in some retail markets, while meat species identification is a major global concern (Murugaiah et al., 2009). In order to protect Muslim consumers from fraud and adulteration, several analytical approaches have been made to identify animal species in food products (Che et al., 2007).

Most analytical methods utilized to date for meat authentication have relied on the detection of species-specific proteins or DNA (Ballin et al., 2009). Today, however, DNA is considered to be the most appropriate molecule for species detection and identification in foods (Singh and Neelam, 2011). Unlike proteins, DNA is relatively stable at high temperatures, meaning that it can be analyzed not only in fresh and frozen food products, but also in processed, degraded and mixed commodities (Lenstra 2003). Additionally, while the presence and characteristics of proteins depend on the tissue type being analyzed, DNA exists and is identical in almost all cells, and the unique variability and diversity afforded by the genetic code permits the discrimination of even closely-related species (Ballin, 2010).

In order to prevent fraud of meat products in national and international markets, regulatory authorities and food processing companies are increasingly vigilant and require a rapid and specific analytical procedures for authentication. Different analytical techniques based on protein analysis have been applied for meat fraud identifications which are time consuming, expensive and not specific enough. In comparison, DNA based methods are fast, inexpensive and more reliable (Jiaqin et al., 2008; Yin et al., 2009). Molecular authentication methodologies based on polymerase chain reaction (PCR) have been developed and successfully applied for species authentication in different meat products (Stamoulis et al., 2010).

The objective of this study was applying PCR method as a sensitive and specific tool to detect adulteration in some raw and processed meat product (luncheon, hot dog, sausage and minced meat) sold in popular areas in Alexandria city with equine meat.

2. MATERIALS AND METHODS

2.1. Sampling:

One hundred sample of meat products including (luncheon, hot dog, sausage and minced meat) 25 of each type were purchased and collected from popular areas in Alexandria City, Egypt. DNA was extracted from each meat sample and stored at –20 ± 1 ℃ until analyzed. Samples of equine species meat (donkey) were used as positive control in PCR reaction.

2.2. DNA Extraction:

DNA was extracted from each meat products sample and positive control sample by the DNeasy protocol provided with animal and Fungi DNA Preparation Kit (Jena Bioscience Cat. No. PP-208S). Extracted DNA were stored at – 20 ºC till used in PCR technique.

2.3. oligonucleotide primers

Two set of oligonucleotide primers specific for detection of equine DNA were synthesized by Metabion Company, Germany (Kesmen et al., 2007). The sequences and description of the primers used in this work are summarized in table (A).

2.4. DNA amplification:

DNA amplification was done in 25 ul reaction volume containing 12.5 ul of 2X Taq PCR mix (i-Taq Cat. no. 25029), 10 PM of each oligonucleotide primers, 5 ul of DNA template and fill up to 25 ul with DNAse and RNAse free water. PCR was carried out in a gene cycler (Perken Elmer model 6900).

The optimized cycle program for PCR using cytochrome b gene primers of denaturation, annealing and extension temperatures was as follow: initial denaturation of 5 min at 94ºC; 35 cycles of 1 minute at 94ºC, 1 minute at 52ºC and 1min. at 72ºC; and final extension step at 72ºC for 5 min (Ilhak and Arslan, 2007).

2.5.Agarose gel electrophoresis

After amplification a 5 ul of the reaction product was mixed with 1 ul of 6X gel loading buffer and fractionated on 1.5% agarose gel electrophoresis at 100V for 30 min. Gel were stained with ethidium bromide and photographed on UV transilluminator (Ilhak and Arslan, 2007). Samples were considered positive when a single band of DNA at 439 bp were evident in the ethidium bromide stained gels compared with the molecular size marker 50-bp DNA ladder (Jena Bioscience Cat. No. M-214). The gels were then photographed using a Polaroid Camera.

Table 1: The sequences of the oligonucleotide primers specific for detection of equine meat.

Primer	Sequences 5-3	Amplified products bp
Equine	5'- GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA- 3'	439 bp
	5'- CTCAGATTCACTCGACGAGGGTAGTA- 3'	

3. RESULTS AND DISCUSSION

Table (2): Incidence of adulteration of different meat products with equine meat

Products	No. of examined samples	Positive samples by PCR	
		No.	%
Luncheon	25	6	24
Hot dog	25	2	8
Sausage	25	16	64
Minced meat	25	14	56

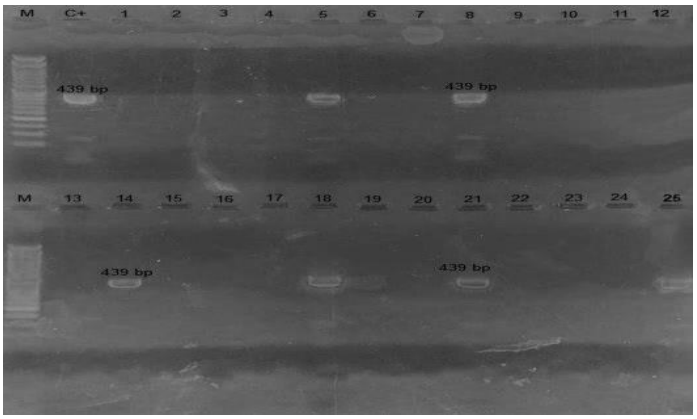

Fig (1): Ethidium bromide stained agarose gel 1.5% electrophoresis showed amplified fragment of 439 bp with species-specific primers. Where, lane M: Molecular marker (50 bp), C+: control positive, lane 5, 8, 14, 18, 21 and 25 positives for equine meat in luncheon.

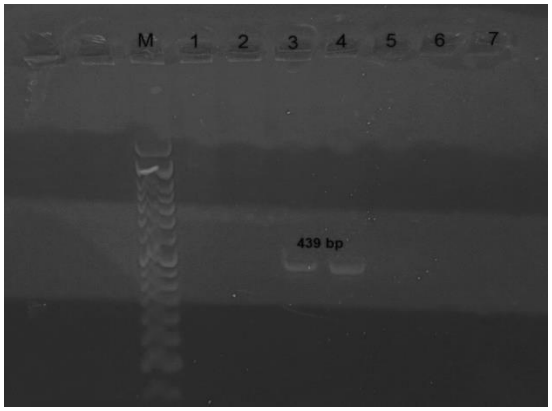

Fig (2): Ethidium bromide stained agarose gel 1.5% electrophoresis showed amplified fragment of 439 bp with species-specific primers. Where, lane M: Molecular marker (50 bp), lane 3, and 4 positives for equine meat in Hot dog.

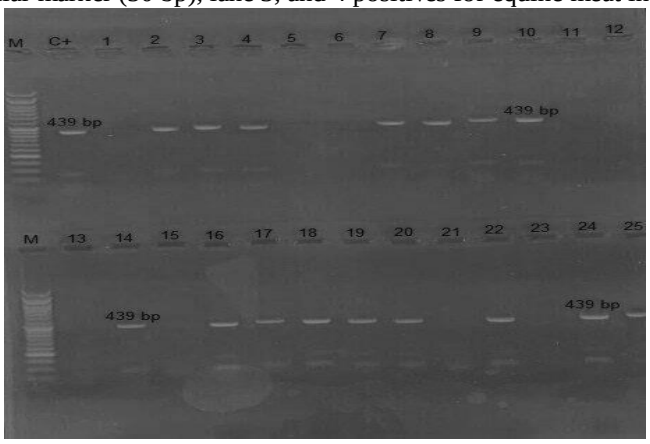

Fig (3): Ethidium bromide stained agarose gel 1.5% electrophoresis showed amplified fragment of 439 bp with species-specific primers. Where, lane M: Molecular marker (50 bp), C+: control positive, lane 2, 3, 4, 7, 8, 9, 10, 14, 16, 17, 18, 19, 20, 22, 24 and 25 positives for equine meat in sausage.

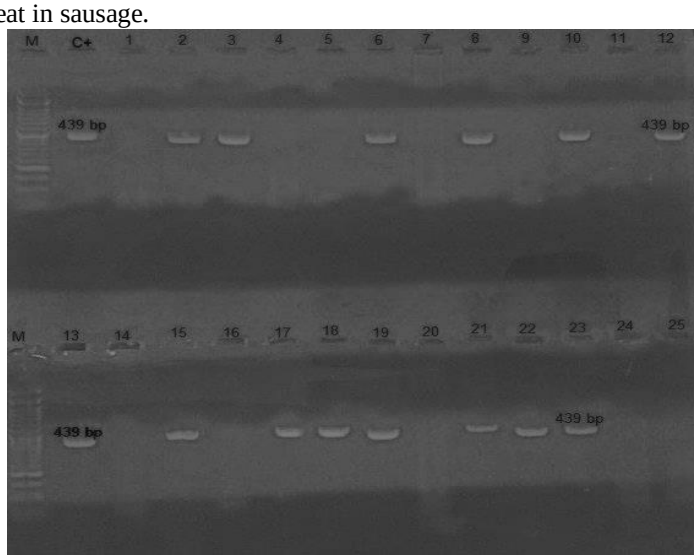

Fig (4): Ethidium bromide stained agarose gel 1.5% electrophoresis showed amplified fragment of 439 bp with species-specific primers. Where, lane M: Molecular marker (50 bp), C+: control positive, lane 2, 3, 6, 8, 10, 12, 13, 15, 17, 18, 19, 21, 22 and 23 positives for equine meat in minced meat.

4. DISCUSSION

Modern molecular techniques, based on DNA analysis, have good applicability in detecting adulteration, and they represent useful complements to methods relying on protein analysis for the identification animal species. DNA-based techniques have become effective and reliable for commercial dairy products also (Feligini et al., 2005). The advantages of DNA-based analysis are manifold. First is the ubiquity of DNA: that from all cell type of an individual contains identical genetic information. Secondly, the information content of DNA is more abundant compared to proteins due to the degeneracy of the genetic codes. Thirdly, DNA is a rather stable molecule which renders DNA extraction and analysis from many sample types feasible (Cheng et al., 2003).

The adulteration by substitution of meat has always been a concern for various reasons such as public health, religious factors, wholesomeness and unhealthy competition in meat market. Of both technological methods of detection of adulteration (protein-based methods and DNA-based methods), the DNA-based methods offer the greatest potential because they are stable and not tissue dependent (Abd El-Azeim et al., 2016).

Results presented in table (2) revealed that the adulteration rate of equine meat in different meat

products were 6 (24%), 2 (8%), 16 (64%) and 14 (56%) in luncheon, hot dog, sausage and minced meats, respectively.

Adulteration with horse meat was previously reported by several investigators (El-Shewy, 2007; Abd El-Nasser et al., 2010; Jaayid, 2013). The adulteration rate with equine meat in our study was more than that reported in Assiut governorate in Egypt by Abd El-Nasser et al., (2010) in minced meat (7%) and sausage (8%); this may be due to no stringent control on food in the popular areas in Alexandria city. Equine is not a commercially used for human consumption. Its presence indicates adulteration for economic gain and so gives an idea that meat has been processed under non-sanitary condition representing potential risks to human health.

The obtained results in Figures (1,2,3 and 4) illustrated that equine meat samples could be detected by agarose gel analysis of PCR products amplified with species-specific primers. A fragment of 439 bp of equine meat was detected in different meat products luncheon, hot dog, sausage and minced meat.

Numerous reports showed that species-specific PCR assay is rapid and cost effective for identification of meat species due to specific detection of target sequence without the need of further sequencing or digestion of the PCR products with restriction enzymes. It is successfully used for identification of various species of meat (Frezza et al. 2008 and Mane et al., 2009). Also, Chisholm et al., (2005) developed real-time PCR assays specific for horse and donkey, applicable for detection of low levels of horse or donkey meat in commercial products.

In meat processing plants, processing more than one species of meat, it may be inevitable that one species of meat may be contaminated with another during meat operations. PCR analysis of such samples may result in positive results for a violation due to its high sensitivity (Sawyer et al., 2003), even though contamination was unintentional and at a very low level. Therefore, precaution should be exercised when interpreting the results of species identification by PCR and analysis of multiple samples should be taken from each lot for an objective evaluation.

5. CONCLUSION

In conclusion, DNA based technological method such as PCR might be useful for effective control of adulterated consumer meat products and violations of labeling requirements for meat products. They are rapid, simple and applicable for detection the meat species in different foods to avoid replacement of animal species by another of lower commercial value or contamination with undesired meat like pig meat.

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